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ABSCISSION:
THE ROLE OF PROTEIN SYNTHESIS

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ABSCISSION: THE ROLE OF PROTEIN SYNTHESIS

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ABSTRACT

Abscission of cotton (Gossypium hirsutum L. var. Acala 4-42) cotyledonary node explants, coleus (Coleus blumei Benth.) nodal explants, and bean (Phaseolus vulgaris L. var. Red Kidney) primary leaf explants was inhibited by the protein synthesis inhibitors dactinomycin and cycloheximide. Stimulated rates of abscission reported by other workers could be due to increased rates of ethylene evolution from treated tissues and their failure to apply the inhibitor close to the separation layer.

During the course of ethylene-enhanced abscission, C^{14} L-leucine incorporation into a trichloroacetic acid-precipitable fraction of tissue homogenates occurred primarily in the separation zone of cotton explants. Little or no enhanced incorporation was observed in the surrounding petiole and nodal tissue. In bean explants, the enhancement was primarily in the pulvinus tissue.

These results support a hypothesis that in abscission the role of ethylene is to stimulate the synthesis of enzymes responsible for the separation of cells in the abscission zone.

1. INTRODUCTION

Enzymatic digestion of pectic materials between cell walls in the separation layer is one of the processes involved in abscission.[1] This enzymatic action represents (i) the movement of preformed protein into the separation layer, (ii) the unmasking of active sites of existing enzymes, or (iii) the biogenesis of the appropriate enzymes.

We will present evidence supporting the hypothesis that de novo synthesis of protein is involved in the separation process. This evidence shows that the protein synthesis inhibitors dactinomycin and cycloheximide prevent abscission, and ethylene enhances incorporation of C^{14} L-leucine into protein.

II. MATERIALS AND METHODS

Four seedlings of either cotton (Gossypium hirsutum L. var. Acala 4-42) or bean (Phaseolus vulgaris L. var. Red Kidney) were grown in soil in 10-cm-diameter pots under 1200 ft-c of fluorescent light with a 14-hr photoperiod at 26 ± 2 C. Cotton explants with 10 mm of the hypocotyl and 3 mm of cotyledonary petioles were isolated from 3-week-old plants. Bean explants from the primary leaf abscission area with 4 mm of pulvinal tissue at the distal side of the separation layer and 6 mm of petiole at the proximal side were isolated from 2-week-old plants.

A clone of coleus (Coleus blumei Benth.) was grown in the greenhouse in 10-cm pots containing soil. During the winter they received 4 hours' additional illumination from 150-watt incandescent bulbs spaced 4 meters apart and 2 meters above the bench. Explants were harvested from plants containing six to eight nodes. Node number one was the uppermost node bearing leaves with petioles longer than 5 mm. Each explant included 3 mm of stem tissue above the node and 10 mm below the node.

Six milliliters of 1.5% agar were poured into 43 ± 2-ml gas-collection bottles (5 cm in diameter and 2.5 cm high) and 10 explants were inserted in the agar so that 3 mm of the explant were submerged. The bottles were fitted with a neck in which a 25-mm-diameter rubber vaccine cap could be placed. Where required, ethylene was injected into the bottles through the vaccine cap to give the specified ethylene concentration. The gas-collection bottles were cut horizontally 1.25 cm from the bottom to facilitate manipulation of the agar containing C^{14} L-leucine and explants. To make the bottles gastight, silicone grease was applied to the groundsurfaces and the bottle halves were held together with adhesive

tape. Control explants were aerated by opening and resealing the gas-collection bottles. The ethylene concentration in the gas phase was monitored by gas chromatography[2] and in the aerated bottles the concentration was not allowed to exceed 0.1 nl ethylene per ml (0.1 ppm) during the experiment. Previous experiments[3] indicate that this amount of ethylene does not accelerate abscission over aerated controls. The bottles were incubated at 25 C under 400 ft-c of continuous fluorescent light (General Electric type 30T8-ww, warm white).

The uniformly labeled C^{14} L-leucine (specific activity 180 mc/mM, 99% biochemically and radiochemically pure) was used as obtained from commercial sources. Agar disks (1.5%) (1.4 mm x 3 mm diameter) containing radioactive leucine were prepared according to the method of McCready.[4] The disks were applied either to the cotyledonary stumps of the cotton explants or to both ends of the bean explant.

The C^{14}-labeled protein was extracted and measured according to a modification of Key's method.[5] Tissue and solutions were kept at 0 to 4 C at all times. Tissue was homogenized in ground glass homogenizers with 1 ml buffer (0.01 M Tris pH 7.5) containing 0.2 mg C^{12} leucine per ml. After two minutes' homogenization, 3 ml of Tris buffer were added and the resultant suspension was passed through glass wool. Samples (usually 3.4 to 3.5 ml) were taken to 5% trichloroacetic acid (TCA) concentration by adding the appropriate amount of 50% TCA, resuspended by shaking, and centrifuged at 3000 x g for 10 min; then the supernatant was poured off. The pellet was resuspended in 5% TCA and the protein was precipitated by centrifugation at 1000 x g for 10 minutes. The precipitate was then suspended in 1 ml of 1 N NaOH containing 0.2 mg C^{12} leucine per ml and 0.1- to 0.2-ml samples were drawn off for protein determination by the Lowery method.[6] The solutions were placed in a 37 C water bath for 10 minutes, chilled, and then made to 5% TCA by adding 50% TCA. The protein solution was centrifuged at 1000 x g for 10 minutes, the supernatant poured off, and the protein resuspended in 5% TCA and centrifuged at 1000 x g for 10 minutes. The pellets were taken up in 2 N NH_4OH and plated in duplicate. The samples were counted on a Tracerlab windowless proportional flow counter. Samples with C^{14} amino acid added at homogenization and prepared in this manner were free of radioactivity.

One microliter of the inhibitors dactinomycin (actinomycin D) or cycloheximide was injected by syringe into explant tissue immediately above the separation layer. Control experiments showed that there was no difference in the rate of abscission between noninjected control explants and those injected with as much as 5 µl of water 1 mm above the separation layer.

III. RESULTS

One method of demonstrating that protein synthesis is an integral part of abscission is by showing that inhibitors such as dactinomycin and cycloheximide[7,8] prevent abscission. Figure 1 shows that dactinomycin is capable of preventing abscission of coleus, cotton, and bean explants. Although not shown, similar data are obtained using cycloheximide as the inhibitor.

Another means of demonstrating a requirement of protein synthesis during abscission is to follow the incorporation of C^{14} L-leucine into proteins of explant abscission zones. If de novo protein synthesis is important, then ethylene, which promotes abscission, should also promote the incorporation of leucine into abscission zone proteins.

Table 1 shows that in the presence of ethylene the enhancement of protein synthesis in cotton explants takes place primarily in the abscission zone of the explant and that there is little or no enhancement in vegetative tissue on either side of the abscission zone.

Table 2 shows that in the presence of ethylene there is a stimulation in the incorporation of leucine into pulvinal proteins of bean explants but little stimulation into the petiole tissue.

The amount of leucine used in these experiments (0.01 ml of 2.8×10^{-8} M) was not high enough to act as an abscission stimulant.[1] Concentrations greater than 10^{-6} M are required for this effect.

IV. DISCUSSION

Our observations with inhibitors of protein synthesis differ from those reported by Lyon and Addicott,[9] who found that dactinomycin and puromycin stimulated abscission. We have observed similar results when the compounds were added in the form of drops to the petiolar stumps of explants of beans, cotton, and coleus or injected at some distance from the separation zone. When, for example, dactinomycin is injected 2 to 3 mm from the separation layer of explants, it is possible to observe both a stimulation (at low concentrations) and inhibition (at higher concentrations) of abscission. We have observed that dactinomycin-treated explants evolve ethylene at faster rates than control explants. For example, 0.1 µg of dactinomycin injected into cotton explants 3 mm above the separation layer raised the ethylene level in the surrounding atmosphere from 0.1 nl ethylene per ml gas phase (0.1 ppm) for controls to

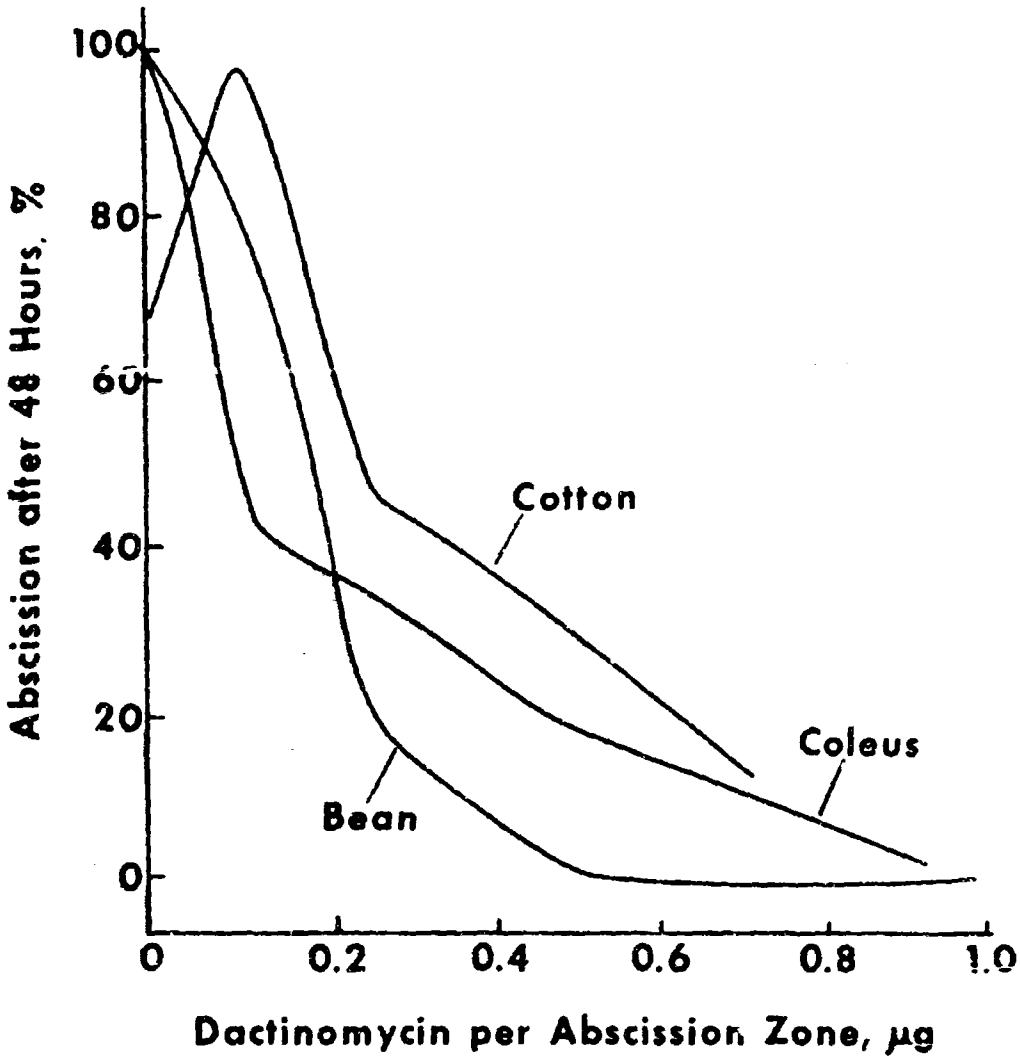
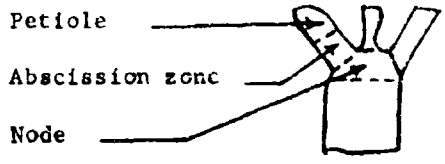


Figure 1. Inhibition of Cotton, Coleus, and Bean Explant Abscission by Dactinomycin. Dactinomycin injected in a 1 µl solution 1 mm above the separation layer.

TABLE 1. EFFECT OF ETHYLENE ON C^{14} L-LEUCINE INCORPORATION INTO COTTON PETIOLE, ABSCISSION ZONE, AND NODE PROTEIN[a]

	10^3 Counts Per Min Per g Fresh Wt			10^3 Counts Per Min Per mg Protein		
	Petiole	Abs. Zone	Node	Petiole	Abs. Zone	Node
Control	237±11[b]	2.79±0.07	0.54±0.06	1,093±42	4.32±0.6	0.75±0.03
Ethylene	260±2	3.89±0.10	0.52±0.06	911±21	7.98±0.2	0.81±0.06
% stim. by ethylene	+10.1	+39.6	-0.03	-7.5	+84.5	+8.0

a.



Agar blocks containing C^{14} L-leucine were placed on the petiolar stumps of cotton explants that were subjected to ethylene (1 nl/ml gas phase) or control atmospheres for 24 hours. The different sections cut from the cotton explants are indicated above.

b. ± standard deviation from an experiment with four replicates; 10 explants per replicate.

TABLE 2. EFFECT OF ETHYLENE ON C^{14} L-LEUCINE INCORPORATION INTO RED KIDNEY BEAN PULVINAL AND PETIOLAR PROTEINS[a]

	10^2 Counts Per Min Per g Fresh Wt		10^6 Counts Per Min Per µg Protein	
	Pulvinus	Petiole	Pulvinus	Petiole
Control	640±8[b]	303±13	1,141±44	890±71
Ethylene	727±13	386±5	1,575±33	920±16
% stim. by ethylene	13.6	27.5	38.1	3.4

a. Agar blocks containing C^{14} L-leucine were placed at the pulvinal and petiole end of bean explants placed on their sides. The explants were subjected to ethylene (1 µl/ml gas phase) and control atmospheres for 24 hours. The ethylene-treated explants had abscissed 92.5% after the 24 hours; the control explants showed no abscission.

b. ± standard deviation from a representative experiment with four replicates; 10 explants per replicate.

0.3 nl for treated explants. These values represent the amount of gas accumulated in the 24-hour period starting 24 hours after the explants were prepared and treated. Earlier experiments (unpublished results) show that this quantity of gas is sufficient to accelerate abscission. Higher concentrations of this inhibitor promoted greater rates of ethylene evolution. However, as the concentration of the inhibitor was increased the rate of diffusion into the site of action was probably also increased and an inhibition of abscission was observed.

A possible interpretation of Lyon's and Addicott's results is that they applied the inhibitors far enough away from the site of action so that the rapid effect of the freely diffusing ethylene masked any potential effect of the slowly diffusing inhibitor. By applying the inhibitor with a microliter syringe within a millimeter of the separation layer, we apparently have been able to circumvent this diffusion barrier.

At this time we do not know how dactinomycin acts to promote ethylene evolution. It is possible that explant tissue is more labile than other vegetative tissue and that ethylene evolution stimulated by dactinomycin may be similar to traumatic responses caused by disease[10] and gamma radiation[11] and represents a nonenzymatic path of ethylene production.

A part of any scheme implicating protein synthesis as an integral part of abscission is showing that ethylene, a potent factor in accelerating abscission, is capable of enhancing protein synthesis where abscission occurs. Our results with cotton indicate that ethylene did, indeed, promote the incorporation of leucine into a TCA-precipitable fraction from the separation zone of cotton cotyledonary explants and that ethylene promoted the incorporation of leucine into pulvinal proteins of the bean explant. Other parts of the cotton explant such as the petiole stump and nodal region failed to show an ethylene-dependent stimulation. These results indicate that the effect of ethylene is not a general one on the protein metabolism of plant tissue but rather is restricted to that area of the explant where ethylene has its physiological effect.

A possible explanation of our results with beans is that during abscission there is a mobilization[12] of amino acids out of the pulvinus, resulting in a decreased pool size in the pulvinus and, in turn, an increased incorporation of the added amino acids. However, in our experiments, C^{14} L-leucine was added to both the pulvinus and petiole and no change was noted in the relative rates of leucine incorporation into the petiole between treated and control explants. If mobilization of amino acids out of the pulvinus was a factor capable of explaining our results, then one would expect a decreased incorporation of leucine into petiole protein.

The ethylene-enhanced leucine incorporation localized distally (in the pulvinus) to the separation as opposed to a few layers of cells on either side of this layer is an unexpected result when compared with that from the cotton experiments. At this time no obvious explanation of the difference is available.

Our results offer evidence supporting the idea that the effect of ethylene in promoting abscission is in part a stimulation of the production of those enzymes integrally associated with the process. However, a conclusive demonstration depends on the characterization of the enzymatic nature of the newly synthesized proteins; for example, whether the proteins are capable of dissolving the pectic material between cells of the separation layer.

The explant assay of abscission offers an interesting example of a possible mechanism in physiological morphogenesis. In an earlier publication[18] we have shown that growth regulators that stimulate ethylene evolution apparently act via a stimulation in production of the enzymes involved in the biogenesis of ethylene. The results presented here indicate that a stimulation of protein synthesis results when certain vegetative tissues are exposed to ethylene. According to our present knowledge, the explant assay may be characterized by the following series of steps: (i) growth regulators act via protein metabolism to accelerate ethylene evolution, and (ii) ethylene in turn acts on protein metabolism to accelerate the synthesis of those enzymes involved in the separation of cells.

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